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Editorial overview: respiratory
Transformational therapies for cystic fibrosis

David N. Sheppard^a, Christine E. Bear^b and Hugo R. de Jonge^c

^aSchool of Physiology, Pharmacology and Neuroscience, University of Bristol, Biomedical
Sciences Building, University Walk, Bristol, BS8 1TD, United Kingdom,

^bPeter Gilgan Centre for Research and Learning, Programme of Molecular Structure and
Function, Hospital for Sick Children, 686 Bay St., Rm. 209420 U-West, Toronto,
ON M5G 0A4, Canada,

^cDepartment of Gastroenterology and Hepatology, Erasmus University Medical Center,
's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands

Address Correspondence to: D.N. Sheppard, Ph.D.
University of Bristol
School of Physiology, Pharmacology and Neuroscience
Biomedical Sciences Building
University Walk
Bristol BS8 1TD
United Kingdom
Tel: +44 117 331 2290
Fax: +44 117 331 1889
E-mail: D.N.Sheppard@bristol.ac.uk

Treatment of the common life-shortening inherited disease cystic fibrosis (CF) is witnessing a revolution. Up until 2012, therapies were directed exclusively against disease symptoms. These included time-consuming chest physiotherapy and small mountain of drugs to clear mucus obstructed air passageways, fight lung infections, dampen inflammatory responses and replace missing pancreatic enzymes. Together with specialised patient care in CF centres, improvements in symptomatic therapy increased average life expectancy from ~10 years in the 1960s to ~40 years in 2010 in North America and Western Europe.

In 2012, ivacaftor (Kalydeco™; VX-770; Vertex Pharmaceuticals), the first drug therapy to target the root cause of CF, mutant cystic fibrosis transmembrane conductance regulator (CFTR) was approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for patients carrying the mutation G551D-CFTR. The clinical impact of ivacaftor was simply astonishing: forced expiratory volume in one second (FEV₁), a blunt measure of lung function, increased by 10% after just 2 weeks in adult patients many with irreversibly damaged lungs; the frequency of hospitalisation for treatment of lung infections, a measure of disease stability, improved by 55%; individuals treated with the drug gained weight (>2.5 kg over 48 weeks) and sweat chloride levels decreased by almost 50 mmol/l, improvements unheard of in previous clinical trials of new therapies for CF. Use of ivacaftor was soon extended to nine other CF mutations, representing ~6% of all individuals living with CF worldwide. This year, use of ivacaftor has been extended to a further 28 mutations, bringing the total to 38 (<https://www.cff.org/News/News-Archive/2017/FDA-Approves-Ivacaftor-for-Five-Splice-Mutations/>). For two reasons, the latest extension of ivacaftor's use is highly significant. First, five of the mutations listed cause splicing defects, which reduce the amount of CFTR protein delivered to the plasma membrane. Second, laboratory data played a pivotal role in expanding the use of ivacaftor to

some rare CF mutations. The willingness of the FDA to consider laboratory data is to be applauded. It raises the exciting possibility that laboratory data might be further employed to expand the clinical utility of ivacaftor and other innovative new therapies for CF.

The development of ivacaftor is notable for several reasons. First, it benefitted enormously from a wealth of basic science research, which identified the *CFTR* gene, demonstrated its function as an ATP-gated epithelial Cl⁻ channel and elucidated how CF-causing mutations disrupt CFTR expression and function. Second, it was assisted by high quality patient registry data and clinical trial networks. Third, the willingness of the CF community to provide help and advice to the Vertex Pharmaceutical team. Finally, the unique collaboration between the US CF Foundation and Vertex Pharmaceuticals, which led to a new model for drug development termed venture philanthropy.

When compared with G551D-CFTR and other mutations that affect only CFTR gating (the pattern of channel opening and closing), transformational therapy for F508del-CFTR, the most common CF mutation is an altogether more challenging proposition because the mutation has multiple impacts upon CFTR. F508del-CFTR is a temperature-sensitive folding mutation. At normal body temperature, the misfolded mutant protein is predominantly retained in the endoplasmic reticulum and degraded by the proteasome. Little if any F508del-CFTR protein traffics to its correct cellular location, the apical membrane of epithelia. The small fraction that does make it forms highly unstable Cl⁻ channels with a pronounced defect in channel gating. Thus, small molecules with two types of activity are required to restore function to F508del-CFTR: first, correctors, so called because they overcome the processing defect of F508del-CFTR and deliver the mutant protein to the apical membrane. Second, potentiators to enhance channel gating following CFTR phosphorylation

by protein kinase A. Combination therapy with lumacaftor (VX-809; Vertex Pharmaceuticals), the first corrector tested in the clinic, and ivacaftor (Orkambi) increased FEV₁ by ~3%, but disease stability by 30-40%, leading in 2015 to regulatory approval for Orkambi use with CF patients homozygous for the F508del-CFTR mutation.

This year's respiratory section of the *Journal* provides a state-of-the-art overview of research to develop transformational therapies for all individual's living with CF. It discusses lessons from clinical trials of ivacaftor and lumacaftor, their mechanism of action and therapeutic strategies under investigation, including mutation-specific schemes to target specific types of CF mutations and mutation-independent stratagems applicable to all CF mutations. The section concludes with discussion of the potential utility of new therapies for CF in the treatment of chronic obstructive pulmonary disease (COPD) and other respiratory diseases.

De Boeck and *Davies* introduce the busy pipeline of therapies being tested in the clinic and highlight the latest clinical trial data to be announced by press release, triple combination therapy with two correctors and a potentiator, which has caused great excitement within the CF community. *De Boeck* and *Davies* draw attention to the ethical dilemma of withdrawing ivacaftor to test new potentiators and the need for better tests than FEV₁ to evaluate lung function, particularly in very young children. They emphasize important lessons from the clinical trials of new therapies conducted to date, including the evaluation of treatment benefit over the long, not short, term, the design of clinical trials to detect unexpected health benefits and the issue of equitable access to transformational therapies for all eligible individuals.

There then follows three reviews on ivacaftor, lumacaftor and combination therapy for F508del-CFTR with correctors and potentiators. These reviews introduce the molecular defects in CFTR targeted by potentiators and correctors, discuss their mechanism of action and highlight the wide spectrum of CF mutations that might benefit from these small molecules.

Jih, Lin, Sohma and Hwang review proof-of-concept studies using ATP analogues and early studies of potentiators identified by hypothesis-led studies prior to the implementation of high-throughput screening to discover efficacious CFTR modulators. Using a sophisticated gating model, the authors explain how ivacaftor potentiates both ATP-dependent and ATP-independent gating of CFTR (Eckford PD *et al. J Biol Chem.* 2012; 287:36639-49; Jih KY, Hwang TC. *Proc Natl Acad Sci U S A.* 2013; 110:4404-9). Of note, *Hwang* and his colleagues draw attention to the therapeutic potential of combinations of potentiators acting at distinct binding-sites to achieve enhanced rescue of mutant channel gating through pharmacological synergy.

Mijnders, Kleizen and Braakman discuss the challenge of correcting CFTR structure with small molecules to repair the defective processing of CF mutants, particularly F508del-CFTR, which not only causes misfolding of the first nucleotide-binding domain (NBD1), but perturbs CFTR domain assembly. They emphasize that CFTR correctors are likely to have wider utility than CF processing mutations because CF mutations that alter Cl⁻ channel function cause structural defects and hence, CFTR misfolding. *Braakman* and colleagues advocate that high-throughput screening is the most practical approach to identify new correctors for rare CF mutations unresponsive to lumacaftor.

Hanrahan, Matthes, Carlile and Thomas argue persuasively that evidence for multiple corrector binding sites provides a strong rationale for rescue of F508del-CFTR with combinations of two or more correctors. The authors draw attention to the problem of drug-drug interactions encountered with lumacaftor and ivacaftor, cautioning that combination therapy increases the potential for drug-drug interactions with potentially far reaching consequences. However, *Hanrahan* and colleagues are optimistic that the development of second generation correctors and corrector combinations will improve significantly the quality of life for most individuals living with CF.

The next two reviews address transformational therapies for individuals with rare CF mutations including missense, nonsense and splicing mutations. As for the preceding reviews, mutation-specific therapies are the focus of most attention. *Oliver, Han, Sorscher and Cutting* discuss the classification of CF mutations, the molecular complexity of individual CF mutations and the challenge of developing precision medicines for all forms of CF. They review the development of ivacaftor, emphasizing the importance of molecular understanding for regulatory approval, but caution that expansion of ivacaftor to very rare CF mutations will require alternatives to conventional clinical trial design. *Cutting* and colleagues advocate an integrated approach combining *in vitro* studies using cell-based model systems with *in silico* predictions and pre-clinical testing to predict the response of CF mutations to correctors and potentiators.

Oren, Pranke, Kerem and Sermet-Gaudelus explain the molecular consequences of nonsense and splicing mutations. They review the development of ataluren, which suppresses premature termination codons (PTCs) caused by nonsense mutations without affecting termination at normal stop codons, discussing potential causes of variable drug

responses, which might have contributed to the drug's failure in clinical trials. Because PTC suppression might introduce the wrong amino acid, leading to a faulty protein, the authors highlight the value of combining PTC suppression with correctors and potentiators. They also draw attention to the therapeutic potential of antisense oligonucleotides (ASOs), chemically-modified synthetic RNA-like molecules to correct nonsense and splicing mutations. Of note, ASO-based therapies have recently received regulatory approval for two genetic diseases, spinal muscular atrophy and Duchenne muscular dystrophy.

In the final review addressing mutation-specific therapies, *Callebaut, Hoffmann and Mornon* discuss the role of structural data in CF drug development. The authors highlight new insight into CFTR structure-function relationships from the first high-resolution structures of CFTR solved by cryo-electron microscopy (Zhang Z, Chen J. *Cell*. 2016; 167:1586-1597.e9; Zhang Z *et al. Cell*. 2017; 170:483-491.e8). They explain how the interface between the NBDs and membrane-spanning domains (MSDs) of CFTR is both a hot spot for CF mutations and a potential binding site for CFTR modulators; other potential drug-binding sites have been identified at the NBD1:NBD2 interface, the location of the two ATP-binding sites that control channel gating (Kalid O *et al. J Comput Aided Mol Des*. 2010; 24:971-91; Hwang TC, Sheppard DN. *J Physiol*. 2009; 587:2151-61). *Callebaut* and colleagues advocate an integrated approach to identify high-affinity CFTR modulators, which combines knowledge from high-resolution cryo-electron microscopy maps and detailed 3D molecular models with information about the conformational stability and flexibility of the CFTR protein required for Cl⁻ channel function.

The next two reviews address two distinct mutation-independent therapeutic strategies both with long histories in CF research. CFTR bypass therapy utilises other pathways for

anion transport to restore transepithelial ion transport to CF epithelia, while gene therapy aims to replace or repair the faulty CFTR gene in CF epithelial cells. Both strategies predominantly target CF lung disease, unlike orally-bioavailable small molecules, which target all affected tissues.

Li, Salomon, Sheppard, Mall and Galletta compare and contrast the localisation, biophysical properties and regulation of the anion channels TMEM16A and SLC26A9 with those of CFTR. Because activation of TMEM16A is transient, the authors explain that long-acting small molecules that directly target TMEM16A are required for bypass therapy. By contrast, the constitutive activity of SLC26A9 makes it a promising target for CFTR bypass therapy. However, co-expression of SLC26A9 with CFTR argues that SLC26A9 potentiators have greatest utility with CF mutants present at the plasma membrane. The authors also introduce an alternative approach to CFTR bypass therapy, self-assembled anion channels and artificial anion transporters developed by supramolecular chemists. They highlight proof-of-concept studies which explore the feasibility of bypassing CFTR dysfunction with artificial anion channels and transporters and consider the challenges that must be overcome to develop these agents as therapeutics for CF.

Hart and Harrison review gene therapy for CF lung disease, a field that has been rejuvenated by rapid advances in gene editing technologies. The authors describe efforts to improve viral vectors, highlighting the important role that new animal models and human intestinal organoids have played in vector testing. They show how DNA nanoparticles have improved the delivery of non-viral gene therapy formulations to mouse lungs *in vivo*, while minicircle DNA vectors have achieved higher levels of gene expression and reduced inflammatory responses. *Hart and Harrison* explain how different gene editing strategies

have been used to achieve gene therapy for CF. They highlight how a “super-exon” has the potential to correct many different CF mutations, eliminating the requirement for mutation-specific gene editing. The authors are optimistic about the prospects of treating CF with gene therapy.

Solomon, Fu, Rowe and Collawn explain how cigarette smoke, a leading cause of COPD results in acquired CFTR dysfunction, characterised by decreased CFTR mRNA, reduced protein stability at the plasma membrane and inhibition of channel gating. They review evidence for acquired CFTR dysfunction in other respiratory diseases, including asthma and non-CF bronchiectasis, concluding that sub-sets of patients in both diseases demonstrate acquired CFTR dysfunction. Of special note, the authors demonstrate that ivacaftor rescues CFTR function after exposure to cigarette smoke and report pilot clinical data showing that ivacaftor improves respiratory symptoms in individuals with COPD. Given the current lack of effective therapies for COPD, these data suggest that ivacaftor and other CFTR modulators have significant therapeutic potential for COPD.

Although the ten reviews provide an excellent overview of work to develop transformational new therapies, some topics have not received the attention they deserve. First, studies of correctors have focused only on pharmacological chaperones that interact directly with misfolded CFTR. An alternative approach is proteostasis regulators, which target one or more of the many CFTR-interacting proteins that orchestrate and control the biosynthesis of CFTR, its delivery to, and expression at the apical membrane (for review, see Balch WE *et al. Science*. 2008; 319:916-9; Amaral MD, Farinha CM. *Curr Pharm Des*. 2013; 19:3497-508). Second, a small number of small molecules have been identified with both corrector and potentiator activity, termed corrector-potentiators or dual-acting small

molecules (Pedemonte N *et al. J Biol Chem.* 2011; 286:15215-26; Phuan PW *et al. Mol Pharmacol.* 2011; 80:683-93). This interesting class of small molecules deserves further investigation, but it is currently unclear whether therapeutically-active dual-acting small molecules will be developed. Finally, advances in stem cell research, with the potential to generate patient-specific epithelial tissues from induced pluripotent stem cells (iPSCs) presents future opportunities for therapy development. Robust protocols for tissue-specific differentiation of patient-derived iPSCs are advancing to the stage where it will soon be feasible to profile a panel of existing and emerging CFTR modulators simultaneously for all affected tissues in an individual to enable precision medicine (Ahmadi S *et al. NPJ Genom Med.* 2017; 2:12). The exciting, longer term goal of tissue or organ replacement with patient-specific cells in which the CFTR mutation is corrected, is being actively pursued by multiple laboratories.

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